

WHAT IS CLAIMED IS:

1. A method for identifying a target epitope comprising screening products of an expression library generated from DNA or RNA derived from a cell expressing the target epitope with cytotoxic T cells generated against the cell to identify DNA clones expressing the target epitope.
2. The method of Claim 1 wherein the target epitope is specific to a cell infected with a virus, fungus or mycobacteria.
3. The method of Claim 1 wherein the target epitope is specific to an autoimmune disease.
4. The method of Claim 1 wherein the expression library is constructed in a viral vector infectious for mammalian cells.
5. The method of Claim 4 wherein the viral vector is constructed by trimolecular recombination.
6. The method of Claim 4 wherein the viral vector is a vaccinia viral vector.
7. A method for identifying a tumor specific target epitope comprising screening products of an expression library generated from DNA or RNA derived from a tumor cell expressing the target epitope with cytotoxic T cells generated against the tumor cell to identify DNA clones expressing the target epitope.
8. The method of Claim 7 wherein the cytotoxic T cells react with tumor cells derived from a non-tumorigenic cell line and do not cross-react with the non-tumorigenic cell line.

9. The method of Claim 7 wherein the cytotoxic T cells are derived from animals tolerized with a non-tumorigenic cell line and are then immunized with tumor cells derived from the non-tumorigenic cell line.

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10. The method of Claim 9 wherein the cytotoxic T cells are derived from animals tolerized with a non-tumorigenic cell line that does not express costimulator activity and are subsequently stimulated with a tumor cell line expressing
10 costimulator activity.

11. The method of Claim 7 wherein the genes expressed in tumor cells are used to generate HLA restricted cytotoxic T cells which are evaluated for activity against tumor cells.

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12. The method of Claim 7 wherein the tumor cell is derived from a single immortalized, non-tumorigenic cell line.

20 13. The method of Claim 12 wherein the screening is performed on a panel of tumor cell lines each derived independently from a single non-tumorigenic cell.

14. The method of Claim 7 wherein the expression
25 library is constructed in a viral vector infectious for mammalian cells.

15. The method of Claim 14 wherein the viral vector is constructed by trimolecular recombination.

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16. The method of Claim 14 wherein the viral vector is a vaccinia viral vector.

17. A method for identifying a target epitope or
35 antigen comprising:

(a) providing cytotoxic T cells specific for a gene product differentially expressed by a cell expressing the target epitope, and

(b) measuring crossreactivity of the cytotoxic T cells for the cell in which target epitopes are identified as the gene product which induces cytotoxic T cells.

18. The method of Claim 17 wherein the target epitope is specific to a cell infected with a virus, fungus or mycobacteria.

19. The method of Claim 17 wherein the target epitope is specific to an autoimmune disease.

20. The method of Claim 17 wherein a modified differential display method is employed that increases resolution of DNA fragments and reduces the frequency of false positives.

21. The method of Claim 20 further comprising use of the DNA fragments to isolate longer gene products following solution hybridization to single strand circles rescued from a phagemid DNA library.

22. A method for identifying a tumor specific target epitope or antigen comprising:

(a) providing cytotoxic T cells specific for a gene product differentially expressed by a tumor cell expressing the target epitope, and

(b) measuring crossreactivity of the cytotoxic T cells for the tumor cell in which target epitopes are identified as the gene product which induces cytotoxic T cells.

23. The method of Claim 22 wherein the tumor cell is derived from a single immortalized, non-tumorigenic cell line.

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24. The method of Claim 22 wherein the assay is performed on a panel of tumor cell lines each derived independently from a single non-tumorigenic cell.

5 25. The method of Claim 22 wherein the generated cytotoxic T cells which react to tumor cells do not react to nontumorigenic T cells.

26. The method of Claim 22 wherein a modified
10 differential display method is employed that increases resolution of DNA fragments and reduces the frequency of false positives.

27. The method of Claim 26 further comprising use of
15 the DNA fragments to isolate longer gene products following solution hybridization to single strand circles rescued from a phagemid DNA library.

28. A viral vector containing a DNA insert flanked by
20 unique sites for restriction enzymes positioned so that religation of the viral vectors arms is prevented and the orientation of the insert DNA is fixed and the DNA insert is operatively associated with a strong regulatory element.

25 29. The viral vector of Claim 28 wherein the vector is constructed by trimolecular recombination.

30 30. The viral vector of Claim 28 wherein the viral vector is a vaccinia viral vector.

B 31. The vector of Claim 28 in which the vector is derived by recombination with plasmid p7.5/tk (SEQ ID NO: 1) or derivatives thereof. ^

B 35 32. The vector of Claim 28 in which the vector is derived by recombination with plasmid pEL/tk (SEQ ID NO: 3) or derivatives thereof. ^

33. A viral vector containing a DNA insert flanked by unique sites for restriction enzymes positioned so that religation of the viral vectors arms is prevented and the orientation of the insert DNA is fixed and the DNA insert is
5 operatively associated with a strong regulatory element wherein the DNA insert encodes a target epitope identified by the method of Claim 1.

34. A viral vector containing a DNA insert flanked by
10 unique sites for restriction enzymes positioned so that religation of the viral vectors arms is prevented and the orientation of the insert DNA is fixed and the DNA insert is operatively associated with a strong regulatory element wherein the DNA insert encodes a target epitope identified by
15 the method of Claim 7.

35. The viral vector of Claim 33 or 34 wherein the viral vector is a vaccinia viral vector.

20 36. A vaccinia viral vector containing a DNA insert flanked by unique sites for restriction enzymes positioned so that religation of the viral vectors arms is prevented and the orientation of the insert DNA is fixed and the DNA insert is operatively associated with a strong regulatory element
25 wherein the DNA insert encodes a target epitope identified by the method of Claim 17.

37. A vaccinia viral vector containing a DNA insert flanked by unique sites for restriction enzymes positioned so
30 that religation of the viral vectors arms is prevented and the orientation of the insert DNA is fixed and the DNA insert is operatively associated with a strong regulatory element wherein the DNA insert encodes a target epitope identified by the method of Claim 22.

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38. The viral vector of Claim 36 or 37 wherein the viral vector is a vaccinia viral vector.

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